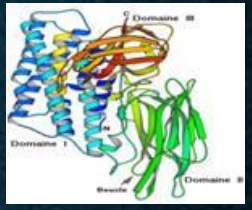
CENTRE DE BIOTECHNOLOGIE DE SFAX



LABORATOIRE DE PROTECTION ET AMÉLIORATION DES PLANTES
****
EQUIPE DES BIOPESTICIDES

Investigation of *Bacillus thuringiensis* Cry1Aa toxicity to *Ephestia kuehniella* (Lepidoptera: *Pyralidae*)

Souad Rouis, Maissa Chakroun, Nouha Abdelmalek and Slim Tounsi

Bacillus thuringiensis

- spore-forming bacteria

- Products crystals during the sporulation phase

- Each crystal is formed by one or more δ-endotoxins (cry proteins) that define the nature of the spectrum insecticidal activity

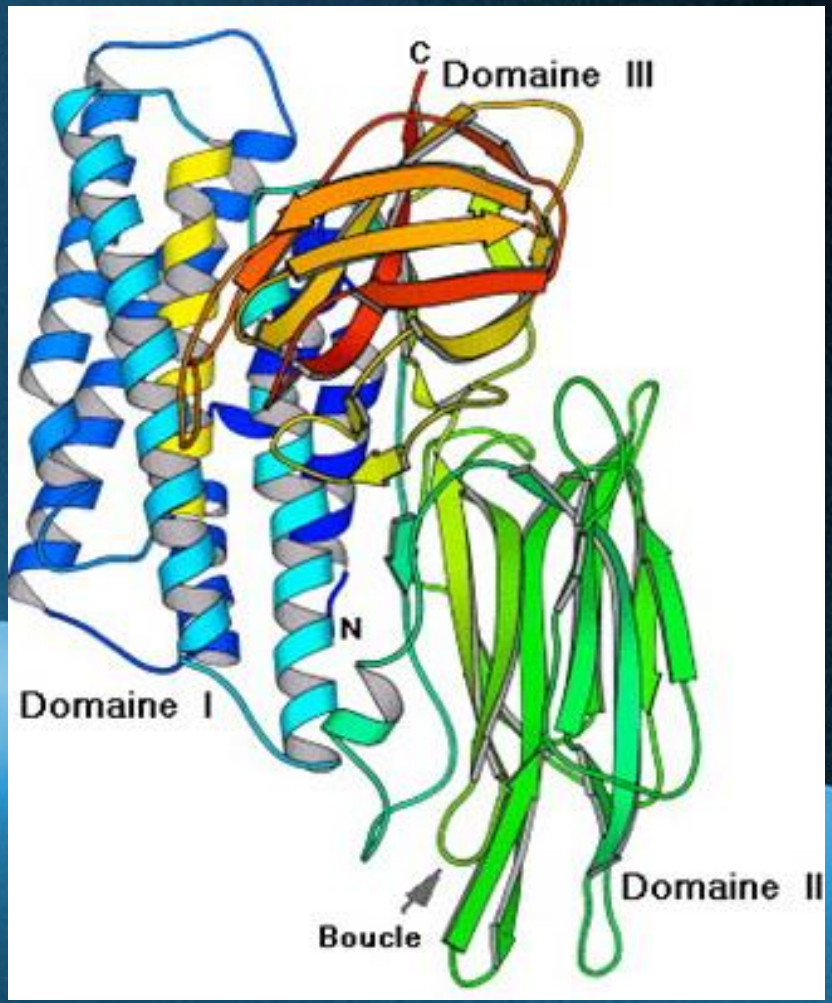
δ-endotoxins Structure

- *3 domains:*

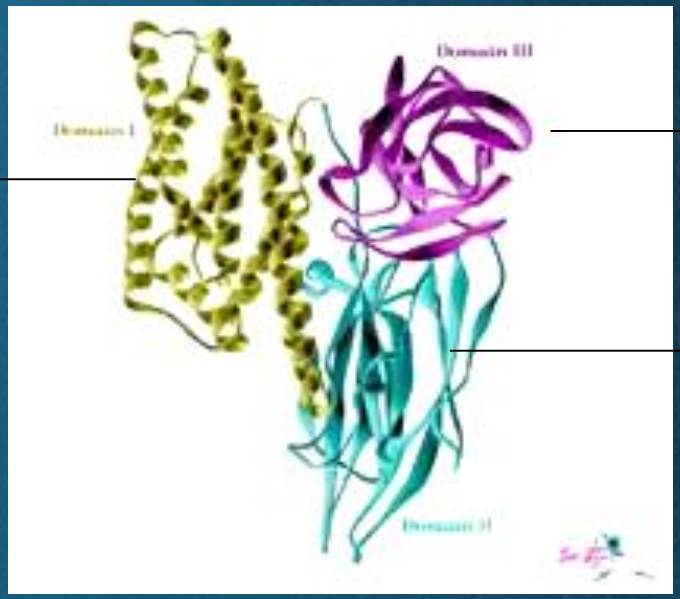
✓ *Domain I: 7 α helices*

✓ *Domain II: 3 β antiparallel feuillets*

✓ *Domain III: 2 β antiparallel feuillets*



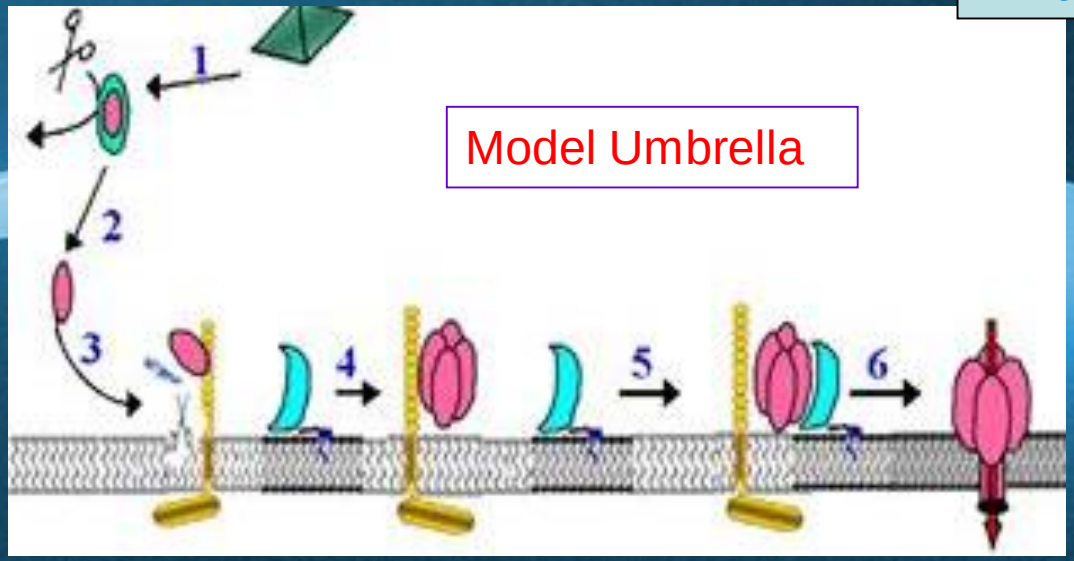
δ-endotoxins Domains specificity



It is supposed to penetrate to the BBM?

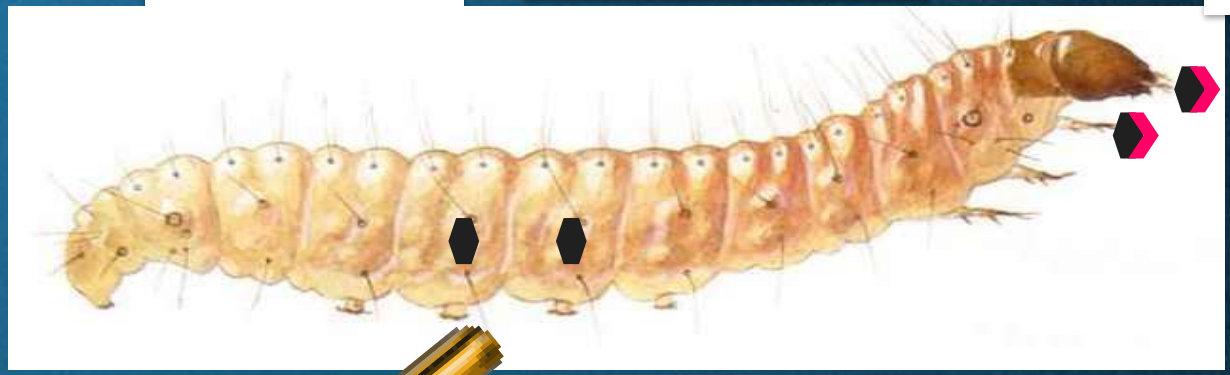
Role is not clear???

It probably interacts with membrane receptors of the intestinal cells



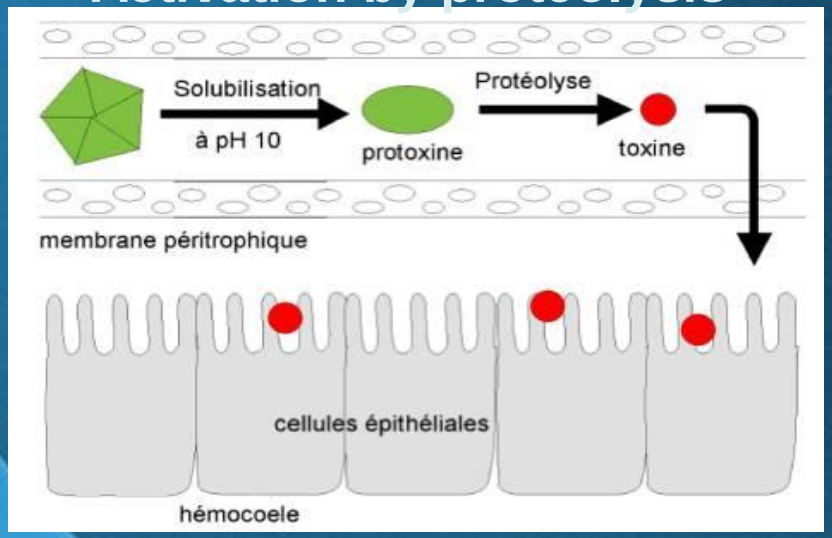
Model Umbrella

Mode of action of δ-endotoxins

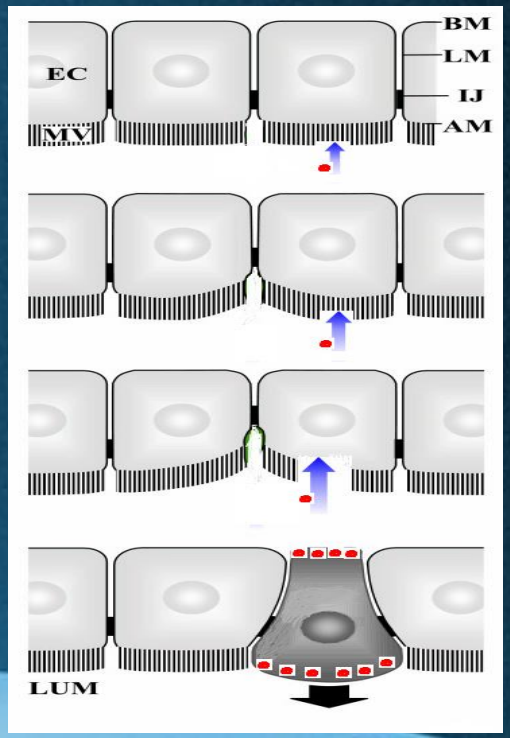


Mode of action of δ-endotoxins

Activation by proteolysis



Toxin-receptor interaction



: Basal Membrane
: Latéral Membrane
: Apical Membrane

The toxin creates pores in the membrane of apical microvilli

Exchange between the intra and extra cellular environment

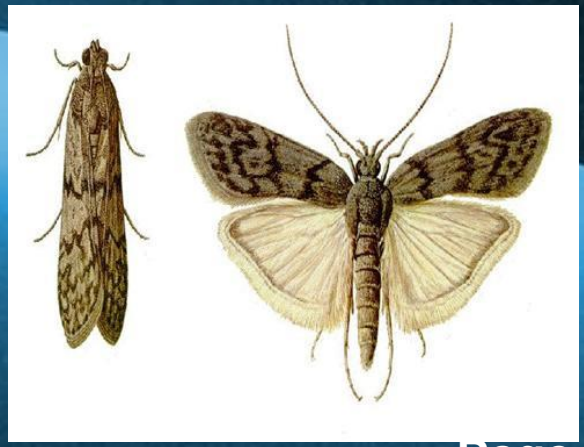
Changes of cell morphology

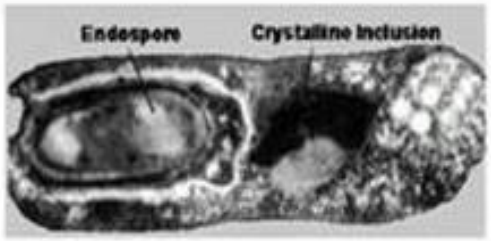
Death of the insect

Cry1Aa protein

	Cry1Ab	Cry1Ac	Cry1Ba	Cry1Ca	Cry1Da	Cry3A	Cry4A	Cry4B	Cry4C
Cry1Aa	90	82	55	67	71	25	27	27	22

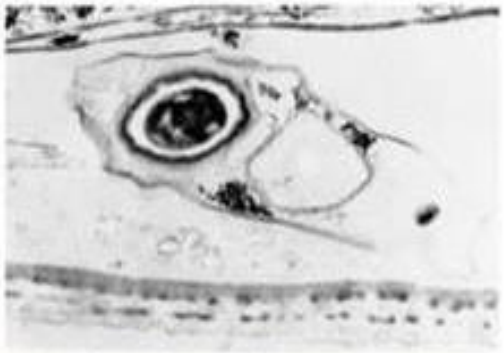
- *Molecular Mass : 130 kDa.*
- *Cry1Aa of BNS3 : Inactive against The flour moth Ephestia kuehniella (Lepidoptera)*





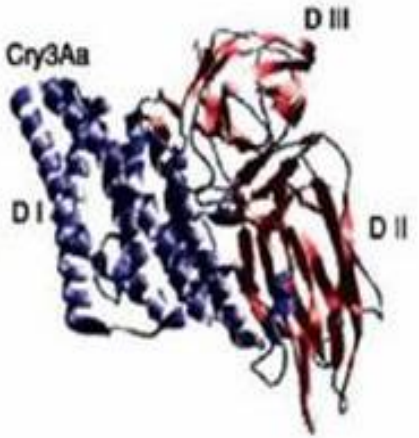
Bacillus thuringiensis (Cry)

Ingestion



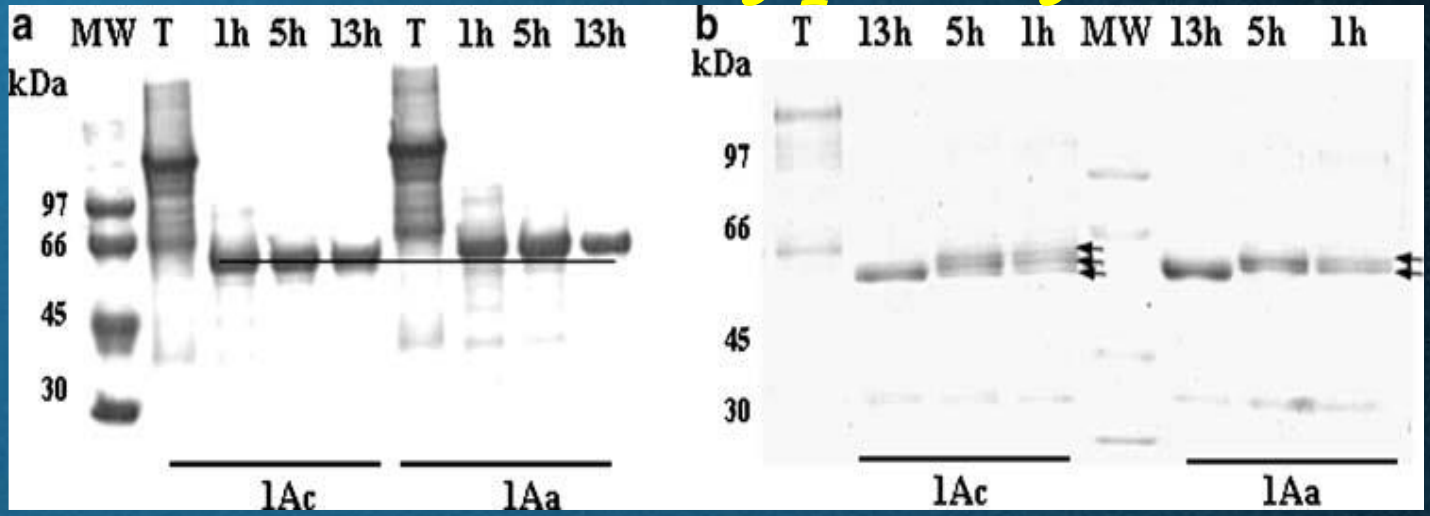
Solubilisation/Activation

Mode d'action



Résistance ?

Impact of the difference of Cry1Aa and Cry1Ac structure on their susceptibility to activation and / or inactivation by proteolysis

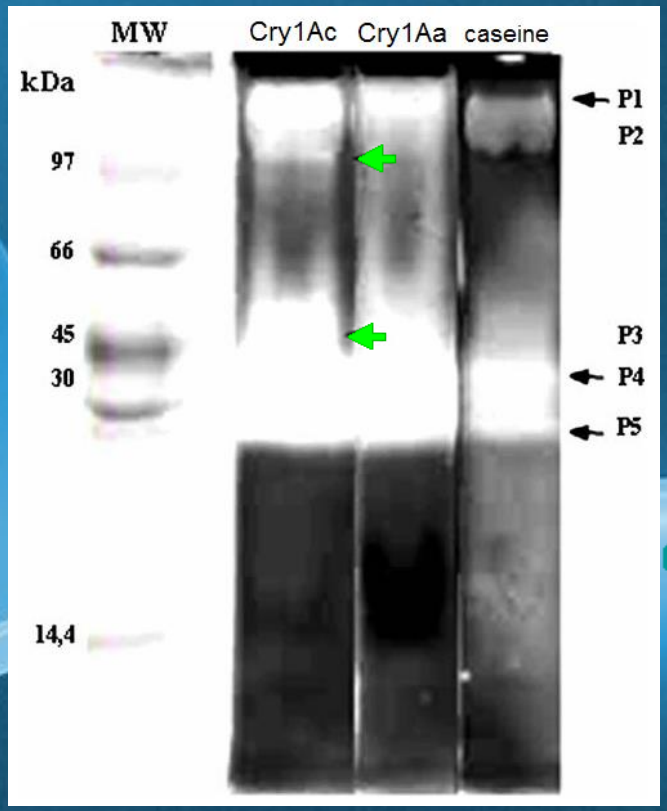


(a) protoxins Cry1Ac and Cry1Aa digested by proteases of the gut juice of E.kuehniella
T: protoxins of Cry1Ac and Cry1Aa

(b) protoxins of Cry1Ac and Cry1Aa digested by the chymotrypsin

- Cry1Ac is fully activated by the proteases of the gut juice of *E. kuehniella*
- Various potential sites for digestion by chymotrypsin

Comparative study by zymogramme of proteases of the gut juice of E. kuehniella involved in the activation of both toxins



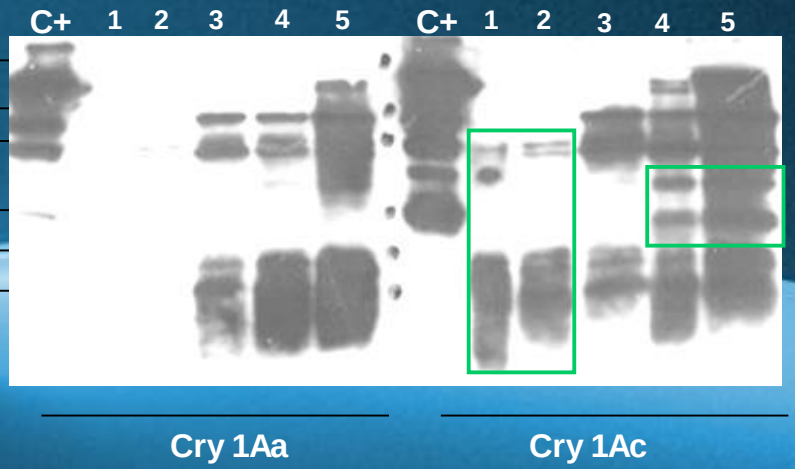
- The protease activity of the juice of *E. kuehniella* (L3) against Cry1Ac involves two additional proteases
- This difference in protease activity could contribute to a relative resistance of *E. kuehniella* towards Cry1Aa

Comparative study of the susceptibility of the two toxins Cry1Aa and Cry1Ac to digestion by Pronase

- The Pronase is a mixture of different types of proteases, serine proteases, metalloproteases, carboxypeptidases, aminopeptidases, chymotrypsin, trypsin

- « Exfashion » Activity : prediction of differences in conformation

1 → 5: decreasing concentrations of Pronase

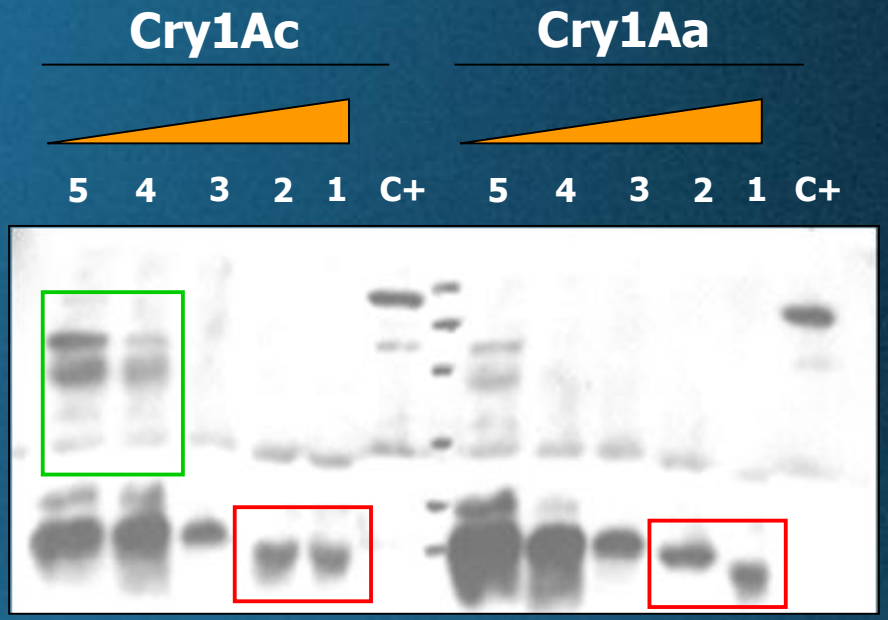


Hybridization with anti-Cry1Aa

Cry1Ac is more resistant to Pronase, suggesting a difference in conformation

Comparative study of the susceptibility of Cry1Aa and Cry1Ac a4-5 domains to digestion by Pronase

Western Blot



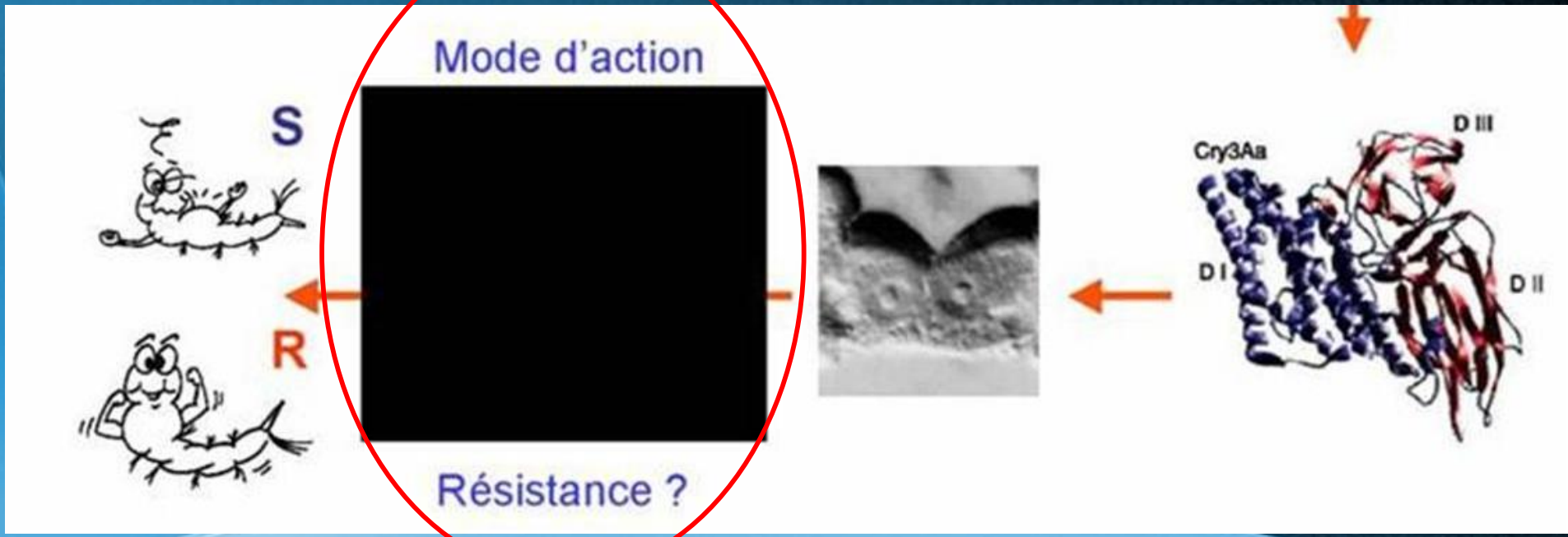
Marqueur de taille
66,2 kDa
45 kDa
31 kDa
20 kDa
14,4 kDa
6,5 kDa

- Cry1Ac α4-5 peptide is more resistant to pronase than Cry1Aa one
- 1- additional cleavage site in Cry1Aa ?

  2- conformational change to protect a cleavage site in Cry1Ac

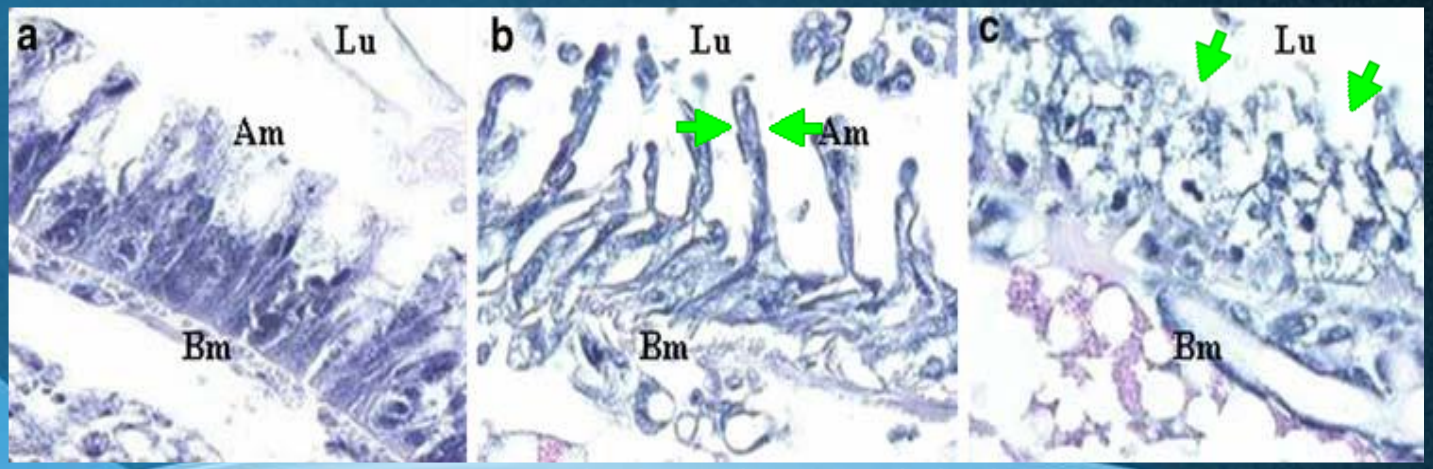
Cry1Ac

Cry 1Aa


Mode d'action
S
R
Résistance ?
Cry3Aa
D III
D I
D II

Histopathological effects of the interaction between Cry1Aa and Cry1Ac with receptors on the intestinal cells of E. kuehniella

control | Cry 1Aa | Cry1Ac



Lumen (Lu); membrane Apicale (Am); membrane Basale (Bm); grossissement 100x

Hypertrophy of Epithelial cells

Complete disintegration of the epithelial cells

Immunohistochemical localization of the toxins Cry1Aa and Cry1Ac in the intestine of E. kuehniella

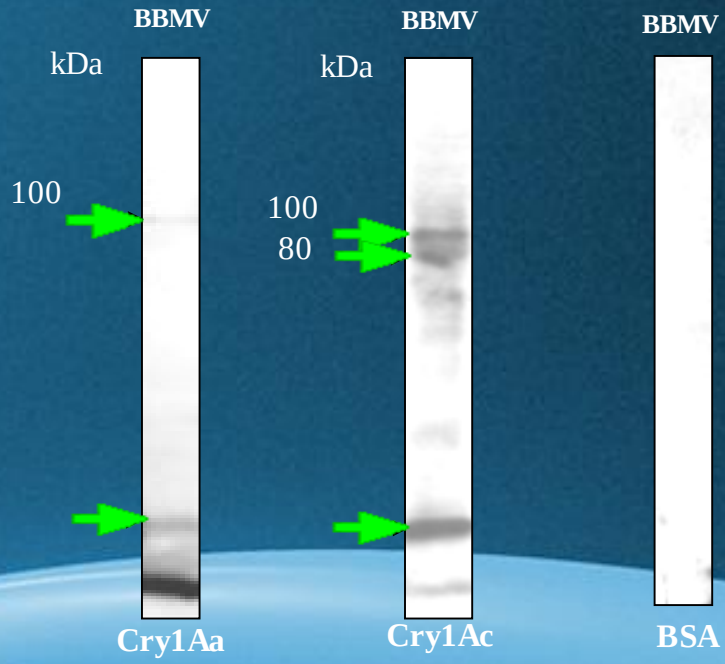
controle | Cry1Aa | Cry1Ac

Lumen (Lu); membrane Apicale (Am); membrane Basale (Bm);Magnification 100x

Slight localization of the toxin Cry1Aa in the apical membrane of epithelial cells

Clear localization of the toxin Cry1Ac in the basal membrane of epithelial cells.

Comparative study « in vitro » of the interaction between the toxins Cry1Aa, Cry1Ac and the receptors of E. kuehniella



- Cry1Ac binds to a number of receptors more important than Cry1Aa
- The intensity of the bands observed in the case of Cry1Ac suggests the presence of a large number of receptors and / or a greater affinity towards the same receptors that Cry1Aa

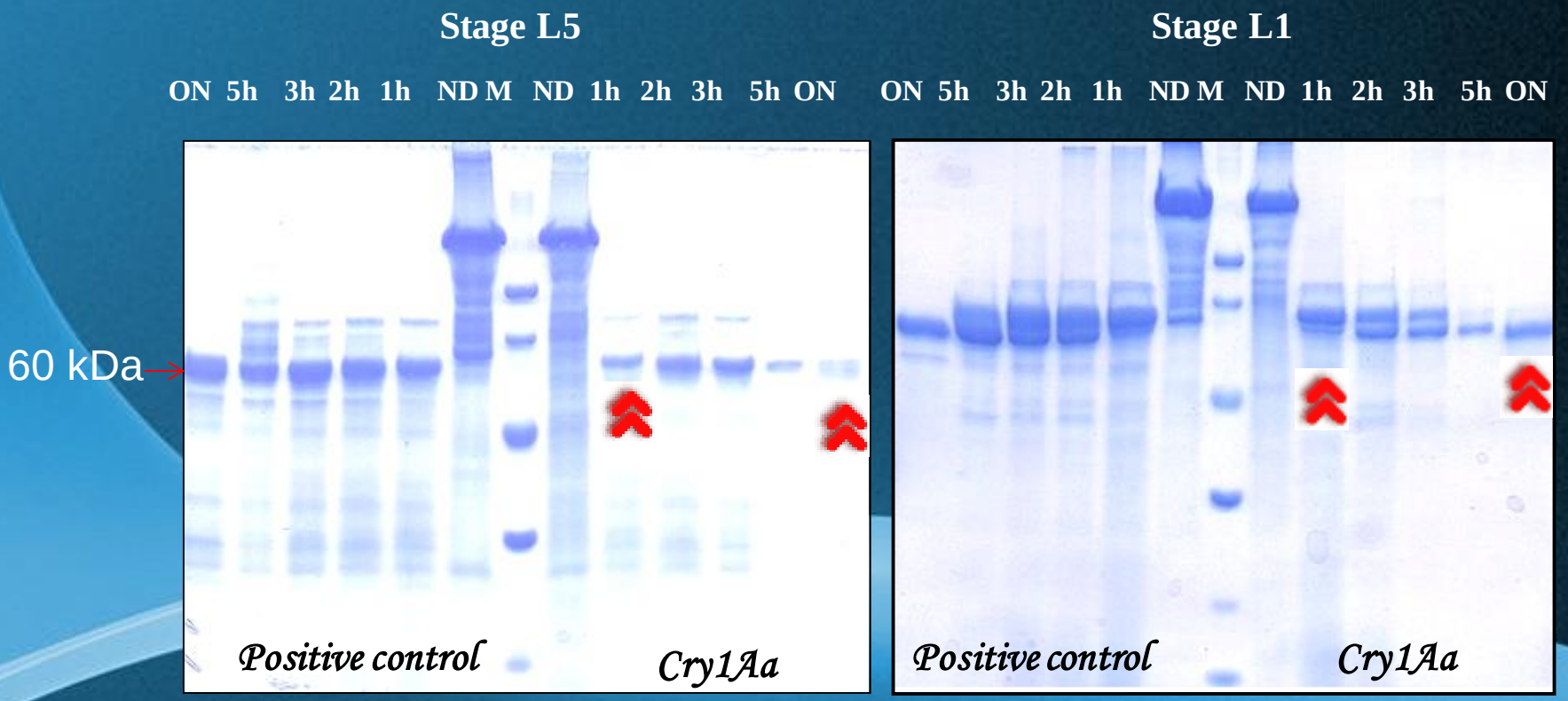

Mode d'action
S
R
Résistance ?
Cry3Aa
D III
D I
D II

Comparative toxicity assay of recombinant strain harboring cry1Aa gene againt Ephestia kuehniella larvea

Positive control	CL50 (µg/g of semolina)
Stage L1	76,148±28,341
Stage L5	90,188±27,308

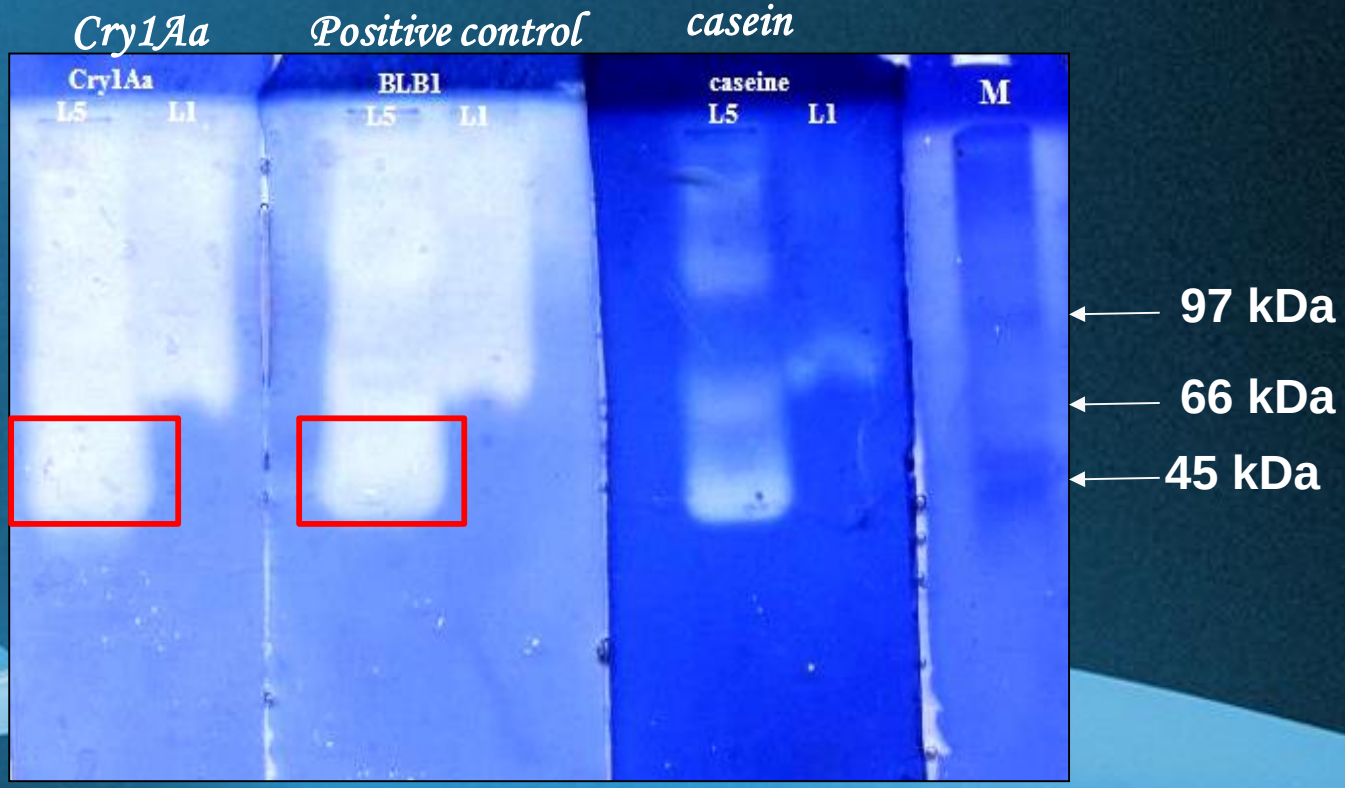
For Cry1Aa, the difference is clear between L1 and L5 larvea stages

Comparative study of the susceptibility of recombinant Cry1Aa to Ephestia kuehniella midgut juce



The battery of proteases, that the insect has, is apparently different from a larval stage to another.

Comparative study of proteases of 1st and 5th instar E.kuehniella larvea gut juice by zymogramme



A clear difference between proteases batteries of 1st and 5th instar E.kuehniella larvea gut juice

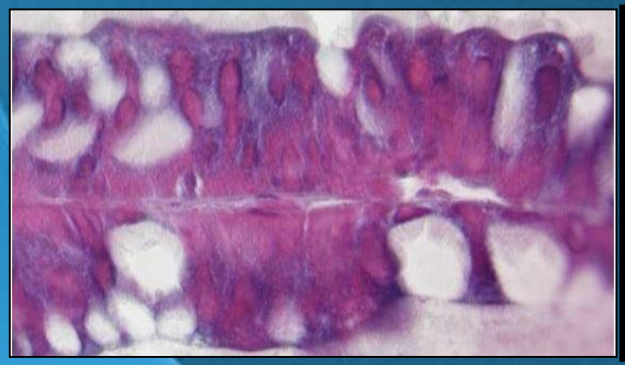
Receptor Investigation of 1st and 5th instar E. kuehniella larvea

SDS PAGE

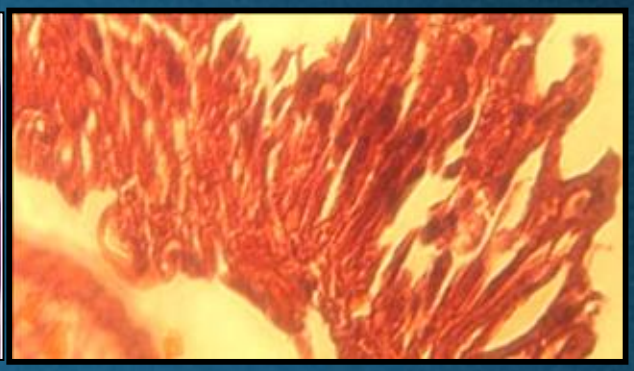
Western Blot

Different receptor profiles between L1 and L5 stages suggesting differences in toxin receptor interaction

Comparative histopathological effects of Cry1Aa on 1st and 5th instar E. kuehniella larvea

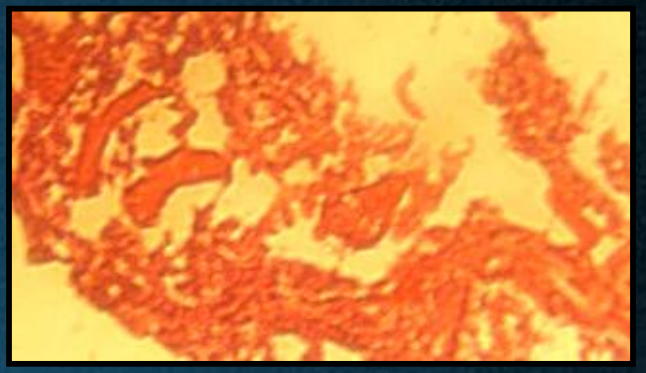


Negative Control



L5

Hypertrophy of Epithelial cells



L1

Apparent disintegration of the epithelial cells

شكرا

Thank you